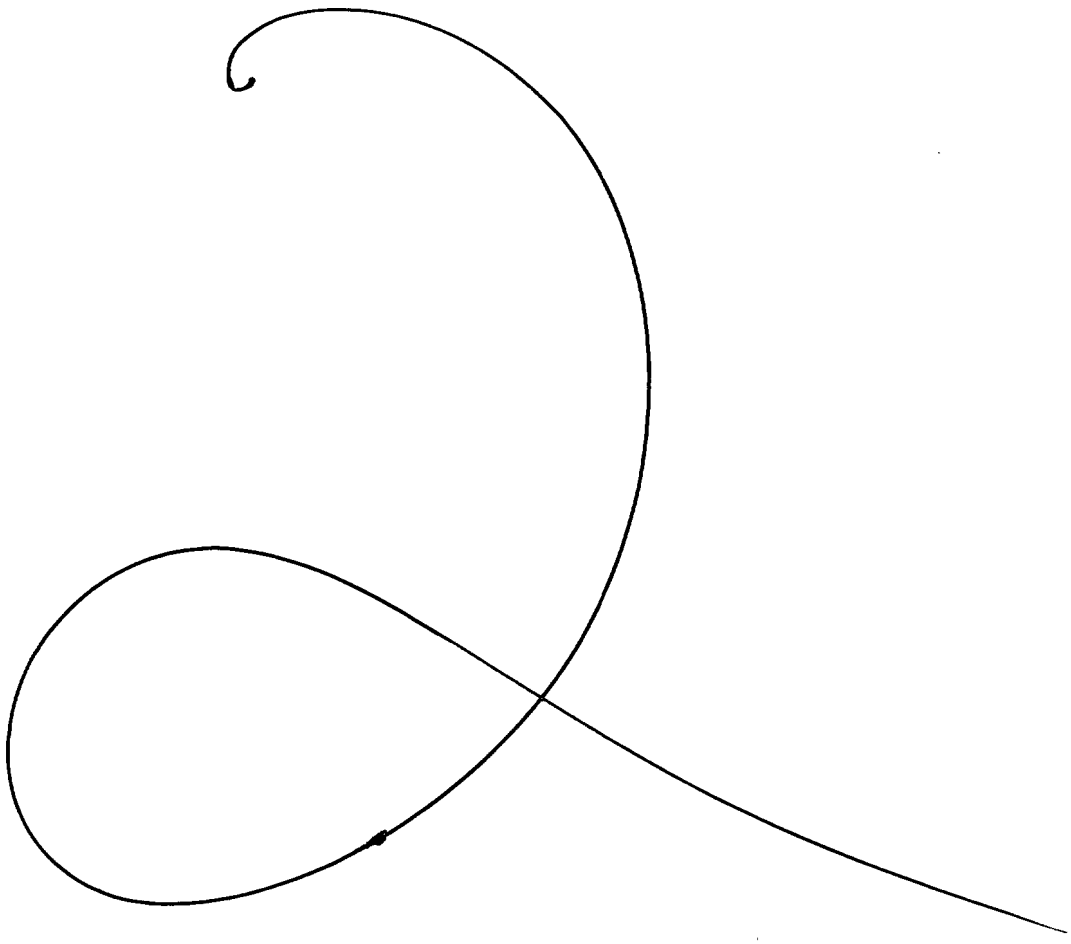


Attorney Docket No. 02036599

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re U.S. Patent Application of:

Jeffrey Owen Phillips

Serial No.: 10/260,132

Filed: September 30, 2002

For: Novel Substituted Benzimidazole Dosage Forms and Composition of Using Same

Examiner: J. Fan

Group Art Unit: 1625

CERTIFICATE OF MAILING BY "EXPRESS MAIL"
"Express Mail" mailing label number EV 113373193 US
Date of Deposit: JULY 18, 2003
I hereby certify that this paper or fee is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" under 37 CFR 1.10 on the date indicated above and is addressed to COMMISSIONER FOR PATENTS, P.O. BOX 1450, ALEXANDRIA, VA 22313-1450

typed or printed name of person mailing paper or fee: TIM HUBALIK

(signature of person mailing paper or fee)

TRANSMITTAL LETTER

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

Enclosed herewith are the following for the above-captioned application:

1. Declaration of Jeffrey O. Phillips;
2. Exhibit 1 – Photograph of May 15, 2003;
3. One copy of three (3) references;
4. Return receipt postcard.

The Commissioner is hereby authorized to charge any additional filing fees required under Rule 1.17 concerning this transaction, or to credit any overpayment to Deposit Account 13-0019.

Respectfully submitted,

MAYER, BROWN, ROWE & MAW LLP

By: _____
Thomas R. Stiebel, Jr.
Reg. No. 48,682

Dated: July 18, 2003

MAYER, BROWN, ROWE & MAW LLP
P.O. Box 2828
Chicago, IL 60609-2828
Telephone: (312) 701-8775

PATENT
Docket No. 02036599

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re U.S. Patent Application of: Jeffrey Owen Phillips	)	
Serial No.: 10/260,132	)	Examiner: J. Fan
Filed: September 30, 2002	)	Group Art Unit: 1625
For: Novel Substituted Benzimidazole Dosage Forms and Composition of Using Same	)	

DECLARATION OF JEFFREY O. PHILLIPS

I, Jeffrey O. Phillips, under penalty of perjury, do hereby state as follows:

1. I am the inventor of the subject matter claimed in the above-captioned patent application.

2. I am employed as Research Associate Professor, Department of Surgery, at the University of Missouri-Columbia, Columbia, Missouri.

3. In this application, I am claiming in pending claims 139 and 216 a powder for suspension and method of treatment using the same. In the recent interview, the examiner raised the following reference in relation to my claims relating to a suspension: Carroll & Trudeau, *Nasogastric Administration of Omeprazole for Control of Gastric pH*, Abstract, 10th World Congresses of Gastroenterology, October 2-7, 1994 (hereafter, "Trudeau").

4. To determine whether a liquid suspension could be created using the method recited by Trudeau for preparing the drug, I obtained the starting materials and performed

Trudeau's method. Such starting materials were one Prilosec® capsule (20 mg omeprazole enteric-coated pellets) and 25 cc of sodium bicarbonate solution having a concentration of 1 mEq/cc. On May 15, 2003, I opened the capsule and crushed the pellets using a mortar and pestle. I then mixed the crushed pellets with the 25 cc of sodium bicarbonate solution in a test tube.

5. Attached hereto as Exhibit 1 is a photograph taken by me on May 15, 2003 of the test tube immediately after I performed Trudeau's method described in paragraph 4 above. As can be seen, a suspension was not formed because the crushed pellets did not disperse or dissolve in the sodium bicarbonate solution as shown in Exhibit 1.

6. In claims 109-134, I am claiming a composition comprising a buffering agent(s) in an amount greater than 10 mEq. As was discussed in the examiner interview, McCullough U.S. Patent No. 5,447,918 discloses at Example 7 a liquid, which contains, among other things, "omerprazole" [sic] 20-300 mg/5ml, calcium carbonate 400-500 mg/5ml, and sucralfate 100-500 mg/5ml. For purposes of this declaration only, I am assuming that McCullough was referring to omeprazole, although this is not clear as "omerprazole" appears throughout the patent.

7. Example 12 of McCullough discloses a tablet with 20-300 mg "omerprazole," 400-500 mg calcium carbonate, and 100-500 mg sucralfate. McCullough includes no information regarding whether the "omerprazole" is enteric coated or, if it is uncoated, whether it is protected from acid degradation in the stomach secretions, or whether sucralfate has any effect on such degradation.

8. As can be seen in the attached reference, Kromer et al., *Differences in pH-Dependent Activation Rates of Substituted Benzimidazoles and Biological in vitro Correlates,*

Pharmacology 1998; 56:57-70, proton pump inhibitors (PPI) such as omeprazole rapidly degrade in acidic environments. For example, at a pH of 1.2, fifty percent of omeprazole degrades within 2.8 minutes. It is therefore important to protect the omeprazole from gastric acid.

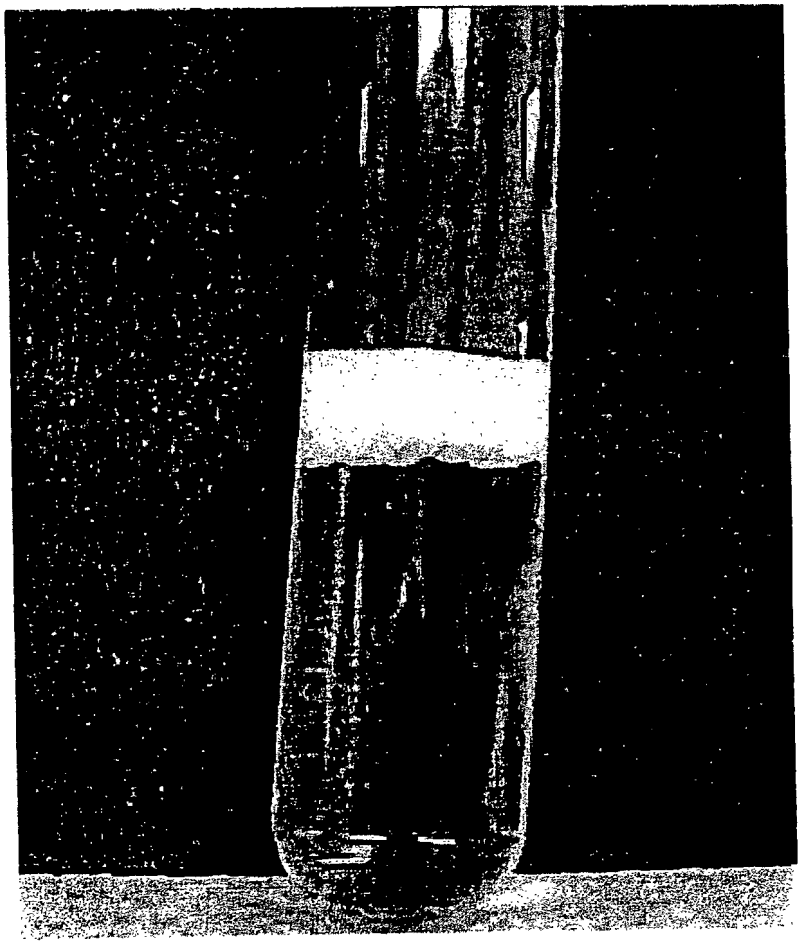
9. As disclosed in McCullough, 500 mg calcium carbonate equates to 10 mEq (i.e., 500 mg divided by molecular weight of 100, which is then multiplied by 2 for the valence of calcium carbonate). However, claim 109 requires greater than this amount.

10. In general, the protective effect of a specific buffer against PPI acid degradation is proportionate to the dose of the buffer employed. See for example, Lin et al., *Evaluation of Buffering Capacity and Acid Neutralizing-pH Time Profile of Antacids,* J. Formosa Med. Assoc. 1998; 97(10): 704-719, copy attached. In figure D on p. 707, 1000 mg (20 mEq) of either calcium carbonate powder or tablets results in an increased pH (i.e., about 5.5 at 5-10 minutes) compared to a dose of 500 mg (i.e., pH of about 1.8 at 5-10 minutes). Combining these results with the data in the Kromer reference shows that omeprazole will be subject to less degradation using 1000 mg than 500 mg. Other examples are provided in figures A-C, and E-F for other antacids.

I declare under the penalty of perjury that the foregoing is true and correct.

Jeffrey O. Phillips

Date: 7/14/2003



10th World Congresses of Gastroenterology
Los Angeles Oct 2 - 7, 1994 Abstracts II: Poster presentations

omeprazole

22P 4970

Title: Nasogastric Administration of Omeprazole for Control of Gastric PH

Matthew Carroll MD, Walter L. Trudeau MD
Division of GI, UC Davis

Acid peptic disease: epidemiology, pathogenesis, diagnosis, treatment >>>

INTRODUCTION: Numerous studies have established the efficacy of prophylactic therapy for the prevention of stress ulceration (SRMD). Maintenance of gastric pH > 4.0 is associated with a decreased incidence of UGI bleeding. Omeprazole is effective in reducing acid secretion, however, currently it is available in delayed-released capsule form only, an not indicated for intravenous or nasogastric administration. Recent reports describe the difficulties with nasogastric tube delivery of omeprazole via NG tube for stress ulceration prophylaxis. METHODS: 9 critically ill patients in the intensive care unit requiring stress ulceration prophylaxis were given omeprazole via NG tube at 20mg every 12 hours. Each capsule was opened, pellets crushed, mixed with 25cc of sodium bicarbonate solution (1meq/ml), and delivered via NG tube. Gastric pH was measured every 6 hours using nasogastric aspirate applied to Gastrocult slides. RESULTS: Patients maintained gastric pH above 5 over 90 % of the time. Gastric pH was maintained above 7 over 65 % of the time. There were no complications throughout the study period. CONCLUSION: Nasogastric administration of omeprazole is effective in controlling gastric pH in the ICU setting. The bioavailability appears to be preserved with the addition of a bicarbonate solution for delivery of the crushed pellets. Nasogastric administration of omeprazole for stress ulceration prophylaxis may prove to be less labor, intensive and more cost effective than IV cimetidine as well as having fewer side effects and drug interactions.

Original Paper

Pharmacology

Pharmacology 1998;56:57–70

Received: June 1, 1997
Accepted: September 16, 1997

W. Kromer[a]
U. Krüger[b]
R. Huber[c]
M. Hartmann[d]
K.W. Steinijans[e]

Departments of
[a] Pharmacology,
[b] Biochemistry,
[c] Pharmacokinetics,
[d] Clinical Pharmacology and
[e] Biometry, Byk Gulden, Konstanz, Germany

Differences in pH-Dependent Activation Rates of Substituted Benzimidazoles and Biological in vitro Correlates

Abstract

Gastric proton pump inhibitors (PPIs) are substituted benzimidazole prodrugs that require an acid-induced activation. Its rate depends on the reactivity of the molecule relative to the environmental pH and determines the drug's tissue selectivity. Factors affecting the exposure of moderately acidic tissues to the activated PPI are the area under the serum concentration-time curve (AUC), serum protein binding, the partition coefficient logP and the serum elimination half-life relative to the chemical activation half-life at a critical tissue pH of about 5. These parameters have therefore been determined in a comparative fashion in the present study. The data shows that pantoprazole is less likely to undergo unwanted activation at moderately acidic targets as opposed to the parietal cell, compared to omeprazole. Actually, although 40 mg pantoprazole (steady state) gave a slightly higher serum AUC of the total parent compound than 40 mg omeprazole (10.5 vs. 7.1 μmol × h × l^{-1}), a higher serum protein binding of pantoprazole versus omeprazole (98 vs. 96%) reversed the AUC values for the free drug in favor of a lower value for pantoprazole (0.19 vs. 0.28 μmol × h × l^{-1}). It is the free parent compound that equilibrates across cell membranes to be activated in acidic tissue compartments. At pH 5.1, the activation half-life of pantoprazole was 4.7 versus 1.4 h for omeprazole, the latter being in the order of the common serum elimination half-life determined in an intraindividual comparison (1.24 vs. 1.25 h). Thus, pantoprazole is eliminated faster from blood than it is activated at a pH of about 5, while omeprazole is as quickly activated at this pH as it is eliminated from blood. Biological in vitro experiments confirmed that pantoprazole displays a lower liability to interfere with unwanted biological targets. This has been demonstrated in vitro for inhibition of both renal Na^+/K^+-ATPase, lysosomal acidification and the production of reactive oxygen species by neutrophils.

Key Words

Proton pump inhibitors
Substituted benzimidazoles
Pantoprazole
Omeprazole
Lansoprazole
Rabeprazole
Tissue selectivity
Prodrugs
Activation rates

KARGER

Fax + 41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

0031–7012/98/0562–0057$15.00/0

This article is also accessible online at:
http://BioMedNet.com/karger

Prof. Wolfgang Kromer, MD
Department of Pharmacology, Byk Gulden
Byk-Gulden-Strasse 2
D–78467 Konstanz (Germany)
Tel. +49 7531 84 2628, Fax +49 7531 84 2413

Introduction

Substituted benzimidazole derivatives like pantoprazole, omeprazole, lansoprazole and rabeprazole are gastric proton pump inhibitors (PPIs) that, after their acid-induced rearrangement to a thiophilic, cyclic sulfenamide, covalently react with SH groups on the extracytoplasmic face of the H^+/K^+-ATPase [for review, see 1–4]. Their tissue selectivities for the secreting parietal cell are based on prodrug accumulation and their activation rates at different tissue pH values, relative to the serum elimination half-life. Apart from steric properties that may contribute to differences in binding patterns of different PPIs at the H^+/K^+-ATPase [4], PPIs probably do not display any target selectivity in terms of a fit between the proton pump and the chemical structure of the drug, as observed with receptor antagonists. However, structural aspects determine the activation rate at different pH values encountered at different (wanted or unwanted) targets. The activated drug then immediately reacts with SH groups in its vicinity irrespective of the particular biological target. As a consequence, drug safety of PPIs requires a sufficient separation between fast chemical activation of the prodrug at pH 1 and an activation rate as slow as possible at pH values above 3 (either 'fast' or 'slow' meant relative to the serum elimination half-life). At the latter pH values, any activation of the prodrug to its pharmacologically active cyclic sulfenamide may produce unwanted effects. For example, pH values of 3–5 have been measured in the attachment zone of osteoclasts and in macrophage lysosomes [5, 6]. Accordingly, Baron et al. [7] have shown that osteoclasts secrete lysosomal enzymes into the acidified extracellular compartment which is considered functionally equivalent to a secondary lysosome with a low pH. Vacuolar acidification also plays a key role in cellular functions like receptor recycling, protein processing and degradation [8].

In order to comparatively assess the potential risk of omeprazole and pantoprazole to produce unwanted SH reactions in the body, their activation kinetics have been determined in vitro at different pH values and compared to their serum elimination half-lives, areas under the serum concentration-time curves (AUCs) values, serum protein binding, volumes of distribution in man and partition coefficients (logP at pH 7.4). Only when all of these parameters are comparatively taken into account can a reasonable judgement be made about the potential tissue exposure to different, activated PPIs at, for example, a critical pH value in the order of 5. The present data suggests that pantoprazole displays a lower liability to produce unwanted SH reactions. We will demonstrate that, in line with their physicochemical properties mentioned above, omeprazole and pantoprazole clearly differ in their in vitro interaction potential at unwanted biological targets, in favor of a lower interference by pantoprazole. The answer to the question of how this basic data translates into a possibly different profile with respect to rare side effects under therapeutic conditions has to await a broader clinical experience over the years to come.

Materials and Methods

pH-Dependent Stability (Activation Half-Life) of Prodrugs

The in vitro conversion (intramolecular rearrangement) from the prodrug to the pharmacologically active cyclic sulfenamide was followed at room temperature at different pH values over time by measuring either the decrease in prodrug concentration or the formation of the cyclic sulfenamide. The initial reaction follows pseudo-first-order kinetics, and the pH-dependent activation half-life was calculated. 2.5–4 mg of the respective prodrug were dissolved in 0.5 ml methanol and diluted to 10 ml with 0.1 *N* HCl (5:95, v/v; pH

1.2) or with b
KH_2PO_4, 0.01
pH of the bu
using HCl or
lowed, at pH
length band o
tration of pro
tometer Perki
ments had al
ide is quantit
pH values fro
by HPLC an
was 8 × 10⁻⁴
Merck-Hitac
(12.5 cm ×
$CH_3CN/10^{-2}$
20% CH_3CN
flow 1 ml/mi

Determir
The logI
according to

Determi
Serum P
Serum fi
–20 °C unti
benzimidaz
232 µmol/l
standard so
equilibrium
lose dialysis
at 37 °C an
(1 ml, 130 r
to pH 7.4 w
sis, radioac
counting (L
furt, Germ
procedures
Dr. P. Eng
many.

Determ
and Ser
40 mg l
The ap
determine
received ir
omeprazol
Pantopraz
quihydrat
the free aci

1.2) or with buffer (mol/l: 0.01 glycine, 0.01 KCl, 0.01 KH_2PO_4, 0.01 H_3BO_3, 0.05 Na-acetate; pH 5–7). The pH of the buffer was adjusted to the desired value using HCl or NaOH. The reaction kinetics were followed, at pH 1.2, photometrically at the long wavelength band of the cyclic sulfenamide (starting concentration of prodrug in this case was 2×10^{-5} mol/l; photometer Perkin-Elmer Lambda 5). Preliminary experiments had already indicated that the cyclic sulfenamide is quantitatively formed under these conditions. At pH values from 5 to 7, reaction kinetics were followed by HPLC analysis (starting concentration of prodrug was 8×10^{-4} mol/l). The HPLC system consisted of a Merck-Hitachi LC 5000 with a reverse-phase column (12.5 cm × 4.6 mm, 5-µm material). The eluant was $CH_3CN/10^{-2}$ mol/l KH_2PO_4 (pH 7.4), the gradient 10–20% CH_3CN in 5 min and 20–70% CH_3CN in 20 min, flow 1 ml/min, UV detection at 280 nm.

Determination of Partition Coefficient

The logP values of the PPIs were determined according to standard procedures [9].

Determination of in vitro Binding to Human Serum Proteins

Serum from 5 healthy male subjects was frozen at –20°C until use. ^{14}C-pantoprazole labeled within the benzimidazole moiety was used at 0.6, 5.2, 47 and 232 µmol/l (final concentrations). Aliquots of drug standard solutions in serum (1 ml) were subjected to equilibrium dialysis (MSE Dianorm apparatus; cellulose dialysis membranes MW 5,000 cutoff, Diachema) at 37°C and 10 rpm against isotonic phosphate buffer (1 ml, 130 mmol/l NaCl, 20 mmol/l NaH_2PO_4 adjusted to pH 7.4 with NaOH, 0.1% NaN_3). After 5 h of dialysis, radioactivity was measured by liquid scintillation counting (Ultima Gold, Packard Instruments, Frankfurt, Germany) and evaluated according to standard procedures [10]. These experiments were performed at Dr. P. Engelmann Menal GmbH, Herbolzheim, Germany.

Determination of Serum Elimination Half-Life and Serum Concentration-Time AUC: 40 mg Pantoprazole versus 40 mg Omeprazole

The apparent terminal half-life and AUC were determined in an open, crossover design. Each subject received in randomized order pantoprazole tablets or omeprazole capsules, in either case 40 mg/day orally. Pantoprazole was administered as the sodium salt sesquihydrate in tablets containing 40 mg referenced to the free acid. Omeprazole capsules containing 20 mg of free compound were purchased from Astra Chemicals GmbH, Wedel, Germany. The medication was protected from light. The daily dose of 40 mg of the respective test drug was administered together with 100 ml tap water in the morning. The study was performed at the Department of Clinical Pharmacology, Byk Gulden, Konstanz, Germany.

26 healthy subjects were selected by history and medical examination including clinical laboratory tests and ECG. Ages ranged from 23 to 43 years (median 30). Body weight ranged from 47 to 97 kg (median 69.5). 24 subjects (13 male, 11 female) completed the study, 1 subject left the study due to an adverse event (common cold infection), the other for personal reasons. Data on AUC and elimination half-life reported here were determined following the 5th oral dose. Blood samples for determination of serum concentrations were taken at 30-min intervals up to 6 h, later at 60-min intervals up to 12 h and, finally, after one additional 12-hour interval.

On day 5 of the study (pharmacokinetic determinations), the volunteers had been fasted overnight (from day 4 to day 5). They had a standard lunch at 12.30 and standard dinner at 7 p.m. Serum concentrations of pantoprazole and omeprazole were determined according to Huber et al. [11] using a validated, reversed-phase gradient HPLC method with UV detection at either 290 nm (pantoprazole) or 301 nm (omeprazole). The detection limit was 0.03 mg/l. The AUC over one dosing interval following the 5th dose and the apparent terminal half-life were calculated by standard methods [12].

Determination of Serum Elimination Half-Life: 40 mg Pantoprazole versus 20 mg Omeprazole

This comparison has been incorporated because it refers to the usual 'standard' doses of the two PPIs. The apparent terminal half-life was determined in a double-blind, randomized, two-period crossover study in 16 healthy male volunteers aged between 21 and 35 years. The study was conducted at the Institute for Clinical Pharmacology, Grünstadt, Germany. Out of a number of parameters measured, only the terminal half-life during steady-state conditions will be reported here. Oral medication was either 40 mg pantoprazole or 20 mg omeprazole once daily for 7 days. The two drugs were administered in identical gelatine capsules. The washout period was at least 2 weeks. Serum concentrations were measured by HPLC, and terminal half-life was determined following the 7th dose. Blood sampling was every 30 min from 0 to 5 h, every hour from 5 to 8 h and, thereafter, 10, 12 and 24 h following

drug administration. Both this study and the study described in the preceding section were carried out according to the German Medicine's Act and were approved by an ethics committee. All volunteers gave their written informed consent.

Determination of Apparent Volume of Distribution

Pantoprazole was administered as an intravenous bolus to 12 healthy male volunteers in a single-blind, randomized, crossover study at doses of 10, 20, 40 and 80 mg. Total serum clearance and apparent volume of distribution were calculated according to standard procedures, as outlined by Bliesath et al. [13]. Omeprazole data was taken from the literature [14]; V_z = volume of distribution, see Notari [15].

Acid Production by Isolated Gastric Glands

Rabbit fundic glands (white New Zealand rabbits, body weight 2–3 kg) were obtained by high-pressure perfusion of the stomach and subsequent collagenase treatment of mucosal pieces. The glands were washed several times and suspended in Krebs-Henseleit solution as described elsewhere [16]. Glands were stimulated with 10 µmol/l histamine for 30 min at 37 °C in the absence or presence of different concentrations of the test compounds. 0.125 µmol/l ^{14}C-aminopyrine was additionally present to accumulate in the acidic space of the parietal cells depending on their activation state. Radioactivity was measured in both the supernatant and the sediment after the incubation period and served as an indirect measure of acid production. A preliminary account of this data was published in abstract form by Simon et al. [17].

Inhibition of Na^+/K^+-ATPase from Dog Kidney in vitro

To assess the potential activity of the different PPIs versus SH-group-containing enzymes other than the gastric H^+/K^+-ATPase, the Na^+/K^+-ATPase from dog kidney (purchased from Sigma, Germany) was measured. 50 mmol/l HEPES buffer of pH 7.6 containing (mmol/l) 3 ATP, 10 $MgCl_2$, 140 NaCl, 10 KCl and 0.5 EDTA was used. Incubation was for 20 min at 37 °C in the absence or presence of the test drug (stock solution in methanol diluted 100-fold by the incubation volume to get the final concentration). The incubation was stopped by addition of 1 ml of a mixture of ammonium molybdate (4.5%; w/v) plus perchlorid acid (60%; w/v) and 3 ml butyryl acetate (100%), cooling to 0 °C and shaking for 10 s. After centrifugation at 2,000 *g* for 5 min, the phosphomolybdate complex was measured photometrically at 310 nm.

Inhibition of Human Polymorphonuclear Leukocyte Function: Formation of Reactive Oxygen Species

Polymorphonuclear leukocytes were isolated from human blood (anticoagulated with sodium citrate) by dextran sedimentation, centrifugation on Ficoll paque and hypotonic lysis of remaining red blood cells [18]. Formation of reactive oxygen species was measured by luminol-enhanced chemiluminescence. The assay was performed in a buffer consisting of (mmol/l) 140 NaCl, 5 KCl, 10 HEPES, 1 $MgCl_2$, 1 $CaCl_2$, glucose 1 mg/ml, BSA 0.05%, luminol 10 µmol/l and microperoxidase 4 µmol/l (all values correspond to final concentrations). Aliquots (0.5 ml) of the cell suspension (10^7 cells/ml) were preincubated for 5 min at 37 °C in the absence or presence of the test drug. Stock solutions (100 mmol/l) of omeprazole and pantoprazole were prepared in DMSO. Final drug concentrations in the assay (1–100 µmol/l) correspond to a DMSO concentration of 0.1% (v/v) which by itself only weakly affected the chemiluminescence response. The assays were transferred into a 'multi-biolumat' LB 9505C from Berthold (Wildbad, Germany) and stimulated by the addition of fMLP (formyl-methionine-leucine-phenylalanine; final concentration 100 nmol/l). Chemiluminescence was continuously recorded for 3 min and the AUCs were calculated. In order to assess nonspecific quenching of chemiluminescence, formation of O_2^- was triggered in the absence of cells under otherwise identical conditions by xanthine oxidase (25 mU) in the presence of hypoxanthine (0.3 µmol/l). The chemiluminescence signal obtained under these conditions was of a comparable magnitude to that seen in the cell system.

Drugs

Histamine (Sigma, Germany); lansoprazole, pantoprazole [19], rabeprazole and omeprazole (for in vitro tests) were prepared by Byk Gulden Chemical Department. Omeprazole for clinical studies was commercially available Antra® (Astra/Hässle). All other chemicals were purchased from commercial sources.

Statistics

As descriptive statistics, either mean ± SD, geometric mean (with geometric 68% range, corresponding to mean ± SD after logarithmic transformation) or median (either 68% range or minimum/maximum) are given. Statistical significances (two-sided, p) were determined using the t test for paired or unpaired samples. The t test was applied in its Welch modification where appropriate. In case of pH-dependent activation of prodrugs in vitro, the coefficients for linear correlation (r) are given.

Results

pH-Depende

The chemic
toprazole, ome
prazole as a m
ty at different
Compared to
pantoprazole
activation hal
cal activation
at pH 5 is mu
half-lives of
rate measurer
vation half-li
zole of 4.7 an
tor of about 3
of pantopraz

Results

pH-Dependent Stability of Prodrugs

The chemical activation half-lives of pantoprazole, omeprazole, lansoprazole and rabeprazole as a measure of their chemical stability at different pH values are shown in table 1. Compared to a favorably slow activation of pantoprazole at pH 5 corresponding to an activation half-life value of 2.8 h, the chemical activation of omeprazole and lansoprazole at pH 5 is much faster, resulting in activation half-lives of 1 and 1.1 h, respectively. Separate measurements at pH 5.1 (fig. 1) gave activation half-lives of pantoprazole and omeprazole of 4.7 and 1.4 h that again differ by a factor of about 3–4 in favor of a slower activation of pantoprazole. For the other PPIs, see ta-

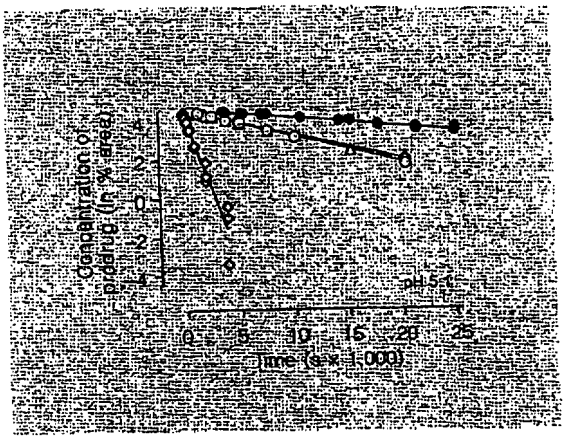


Fig. 1. Concentration of prodrugs over time (i.e. activation kinetics) at pH 5.1 in vitro (ln; % of area under the control HPLC peak). ● = Pantoprazole, r = 0.99; ○ = omeprazole, r = 0.99; △ = lansoprazole, r = 0.99; ◇ = rabeprazole, r = 0.97; n = 40, 29, 30 and 25, respectively; r = coefficient of linear correlation.

Table 1. Chemical activation and serum elimination half-lives of PPIs

	Pantoprazole	Omeprazole	Lansoprazole	Rabeprazole
Chemical activation half-lives				
pH 1.2	4.6 min (4.3; 4.4; 4.7; 5.0)	2.8 min (2.5; 2.7; 2.8; 3)	2.0 min (1.9; 2.0; 2.0; 2.2)	1.3 min (1.1; 1.1; 1.1; 1.7)
pH 5.0	2.8 h (2.5; 2.8; 2.8; 3.2)	1.0 h (0.93; 1.1)	1.1 h (1.1; 1.1)	
pH 5.1	4.7 h (4.1; 4.4; 4.8; 5.5)	1.4 h (1.4; 1.4; 1.5)	1.5 h (1.4; 1.6; 1.6)	0.12 h (0.09; 0.13; 0.15)
pH 6.0	21 h (19; 23)	7.3 h (7.1; 7.5)	6.4 h (6.2; 6.5)	
pH 7.0	73 h (73; 73)	39 h (38; 39)	35 h (33; 36)	
Serum elimination half-lives, h				
	1.24 (0.76; 2.03)	1.25 (0.86; 1.82)	1.4–2.7	1.49 ± 0.78

Chemical activation half-lives were calculated by means of linear regression (fig. 1) during the initial reaction period of at least one half-life (until subsequent chemical reactions may cause some deviation; not shown). Means and single values.

Serum elimination half-lives have been determined under the following conditions: pantoprazole 40 mg/day, following the 5th oral dose in a crossover comparison to 40 mg/day omeprazole (geometric means and geometric 68% ranges); lansoprazole 30 mg/day, following the 7th oral dose (data taken from Barradell et al. [23]); rabeprazole 40 mg/day, following the 7th oral dose, mean ± SD (data taken from Yasuda et al. [55]).

ble 1. By contrast, at pH 1.2, encountered within the acidic canaliculus of the parietal cell, the activation half-lives of pantoprazole, omeprazole, lansoprazole and rabeprazole were within a fairly low range of 4.6–1.3 min. At the other extreme, at pH 7.0, they were 73, 39 and 35 h, for pantoprazole, omeprazole and lansoprazole, respectively. For comparison, pantoprazole and omeprazole displayed activation half-lives of 130 and 80 h, respectively, at pH 7.4 [20].

Partition Coefficient

logP was comparatively determined for pantoprazole, omeprazole, lansoprazole and rabeprazole. At pH 7.4, pantoprazole showed the lowest logP of 2.05 ± 0.01 (mean ± SD, n = 7, table 2). Omeprazole and rabeprazole produced logP values of 2.27 (2.27, 2.27, n = 2, table 2) and 2.3 ± 0.04 (mean ± SD, n = 4). The highest logP value was found for lansoprazole, i.e. 2.8 (2.8, 2.8, n = 2).

In vitro Binding to Human Serum Proteins

Pantoprazole, omeprazole and lansoprazole were directly compared at concentrations of either 0.5 or 3 μg/ml (n = 5 each). Percentages of bound drug (mean ± SD) were 98.2 ± 0.19 (pantoprazole), 96.1 ± 0.54 (omeprazole) and 97.3 ± 0.69 (lansoprazole) for 0.5 μg/ml. The higher concentration of 3 μg/ml gave percentages of bound drug (mean ± SD) of 97.9 ± 0.2 (pantoprazole), 95.4 ± 0.75 (omeprazole) and 97.3 ± 0.33 (lansoprazole). No significant differences between percentages of bound material observed in the comparison between the two concentrations of either drug were found. Note that 0.5 μg/ml of either pantoprazole or omeprazole equal 12 and 15 μmol/l corresponding, roughly, to the upper limits of maximum serum concentration shown in table 2. The percentage of free drug (that penetrates across cell membranes and accumulates in acidic compartments) was significantly different between pantoprazole and omeprazole (1.8 ± 0.19 vs. 3.9 ± 0.54, mean ± SD; $p \leq 0.001$; total in vitro concentration = 0.5 μg/ml). The difference between pantoprazole and lansoprazole (2.7 ± 0.69) was also statistically significant ($p \leq 0.05$).

Serum Elimination Half-Life and AUC: 40 mg Pantoprazole versus 40 mg Omeprazole Once Daily over 5 Days

When directly compared in a multiple-dose, crossover study in 24 healthy volunteers, serum elimination half-lives (geometric means with geometric 68% ranges) of 1.24 (0.76, 2.03; pantoprazole) and 1.25 (0.86, 1.82; omeprazole) were obtained (table 1). The AUC values over one 24-hour interval were 10.5 (5.2, 21.1) and 7.1 (3.2, 15.8) μmol × h × l^{-1}, respectively (table 2).

Serum Elimination Half-Life: 40 mg Pantoprazole versus 20 mg Omeprazole

In a further crossover study in 16 healthy volunteers, oral daily doses of 40 mg pantoprazole and 20 mg omeprazole were compared. Paired samples were available for 14 subjects and were used for evaluation. Following the 7th dose, median serum elimination half-lives (with 68% ranges) of 0.69 h (0.6, 0.98; pantoprazole) and 0.64 h (0.47, 0.71; omeprazole) were determined.

Apparent Volume of Distribution

The volume of distribution is the only parameter presented here that was not determined in a direct comparison between pantoprazole and omeprazole. However, both sets of data refer to 40 mg i.v. (pantoprazole n = 12, omeprazole n = 10) and mean ± SD. The volume of distribution was 0.15 ± 0.02 l/kg (pantoprazole) and 0.37 ± 0.16 l/kg (omeprazole; data taken from Andersson et al. [14]).

Acid Pr
Glands
When g
histamine,
ric means
pantopraz
zole; n = 4
n = 4).

as significant-
ole and ome-
).54, mean ±
ncentration =
:en pantopra-
.69) was also
;).

and AUC:
' mg
5 Days
a multiple-
:althy volun-
:s (geometric
ıges) of 1.24
1.25 (0.86,
ed (table 1).
ıour interval
!, 15.8) μmol

40 mg
ıeprazole
n 16 healthy
0 mg panto-
were com-
ilable for 14
:ion. Follow-
elimination
0.69 h (0.6,
(0.47, 0.71;

)n
is the only
s not deter-
ween panto-
:r, both sets
antoprazole
ıean ± SD.
!.15 ± 0.02
0.16 l/kg
ersson et al.

Table 2. The basis of potential exposure of moderately acidic tissue compartments to activated PPIs in man

Parameter	Pantoprazole	Omeprazole
Maximum serum concentration (40 mg p.o.; steady state), μmol/l	6.2 (3.5, 11.2) n = 24	2.5 (1.4, 5.0) n = 24
Serum AUC (40 mg p.o., steady state), μmol × h × l^{-1}	10.5 (5.2, 21.1) n = 24	7.1 (3.2, 15.8) n = 24
0.5 μg/ml total concentration		
Serum protein binding, %	98.2±0.19*	96.1±0.54*
Free drug, %	1.8±0.19*	3.9±0.54*
	n = 5	n = 5
AUC of free drug (calculated from the above data), μmol × h × l^{-1}	0.19 (0.09, 0.38)	0.28 (0.12, 0.62)
3 μg/ml total concentration		
Serum protein binding, %	97.9±0.2*	95.4±0.75*
Free drug, %	2.1±0.2*	4.6±0.75*
	n = 5	n = 5
Volume of distribution (40 mg i.v.), l/kg	0.15 (0.13, 0.17) n = 12	0.37±0.16* n = 10
logP at pH 7.4	2.05±0.01* n = 7	2.27 (2.27, 2.27) n = 2
Activation half-life at pH 5.1 in vitro, #h	4.7±0.61* n = 4	1.4±0.06* n = 3
Serum elimination half-life (40 mg p.o., steady state), #h	1.24 (0.76, 2.03) n = 24	1.25 (0.86, 1.82) n = 24

Omeprazole volume of distribution from Andersson et al. [14]. For pharmacokinetic characteristics, geometric means and geometric 68% ranges are given unless otherwise stated.

* Mean ± SD.

\# Data from table 1 repeated here for a direct comparison to the other parameters.

Acid Production by Isolated Gastric Glands

When gastric glands were stimulated with histamine, IC_{50} values were (μmol/l; geometric means with 68% ranges) 0.63 (0.5, 0.79; pantoprazole; n = 4), 0.2 (0.17, 0.24; omeprazole; n = 4), and 0.4 (0.26, 0.6; lansoprazole; n = 4).

Inhibition of Na^+/K^+-ATPase from Dog Kidney in vitro

The IC_{50} values (μmol/l; geometric means with 68% ranges) were 200 (145, 275; pantoprazole; n = 6), 32 (18, 56; omeprazole; n = 7) and 40 (31, 51; lansoprazole; n = 5). Hence, pantoprazole proved to be least potent in inhibiting the sodium pump from dog kidney in vitro: $p \leq 0.001$ (pantoprazole vs. omeprazole) and $p \leq 0.001$ (pantoprazole vs. lansoprazole).

Table 3. Selectivity of substituted benzimidazoles in vitro

A Inhibition of acid versus sodium pump (IC_{50}; µmol/l; geometric mean)

	Acid pump		Sodium pump		Selectivity factor
Pantoprazole	0.6	n = 4	200	n = 6	~330
Omeprazole	0.2	n = 4	32	n = 7	~160
Lansoprazole	0.4	n = 4	40	n = 5	~100

B Inhibition of lysosomal acidification (IC_{50}; µmol/l; mean ± SE)

Pantoprazole	194±90	n = 6
Omeprazole	75±26	n = 6

C Inhibition of chemiluminescence of fMLP-stimulated human polymorphonuclear leukocytes (IC_{50}; µmol/l)

Pantoprazole	no inhibition up to 100 µmol/l	n = 5
Omeprazole	~100 (see text)	n = 5

(A) Acid pump: rabbit fundic glands stimulated by histamine; a preliminary account of this work was published in abstract form by Simon et al. [17]. Sodium pump: Na^+/K^+-ATPase from dog kidney. (B) Rat kidney lysosomes in vitro. Data from Simon et al. [16].

Inhibition of Human Polymorphonuclear Leukocyte Function: Formation of Reactive Oxygen Species

Concentrations of 10^{-6}, 10^{-5} and 10^{-4} mol/l of either omeprazole or pantoprazole were tested for potential inhibition of the formation of reactive oxygen species by polymorphonuclear leukocytes as measured via chemiluminescence. Pantoprazole did not display any significant inhibition at all of these concentrations. Although omeprazole was without any effect at 10^{-6} and 10^{-5} mol/l, it caused about 53% inhibition in polymorphonuclear leukocytes ($p \leq 0.01$, two-tailed paired t test) and about 24% inhibition of hypoxanthine/xanthine oxidase in a cell-free system ($p \leq 0.01$, two-tailed paired t test) at 10^{-4} mol/l. The latter value probably represents antioxidative properties of omeprazole (see Discussion). The difference of about 29% between the two systems may indicate the degree of inhibition of the formation of reactive oxygen species by omeprazole, although the different parameters used for this comparison make any quantification difficult. No inhibition of chemiluminescence has been found with 10^{-4} mol/l pantoprazole (table 3).

Discussion

The Antisecretory Potencies as a Basis of Comparison between Substituted Benzimidazoles

Pantoprazole [21], omeprazole [22], lansoprazole [23] and rabeprazole (E-3810 [24]) are substituted benzimidazole sulfoxides that have essentially the same mechanism of action although they display distinct differences [3, 4, 25, 26]. The present paper focuses on pantoprazole and omeprazole and, therefore, their relative antisecretory potencies should be briefly discussed. Kromer et al. [27, 28] had already shown that, overall, the two drugs

proved to b
ments. This
al. [29] and I
unteers com
upon oral
conditions.
zole were les
zole during
over pH m
[32] confirm
20 mg omej
Therefore,
data on pa
been prima
oral dose of
omeprazole
countries ir
zole 40 mg
meantime a
cant advan
in patients
ulcers [33–

*The Imp
Activatic
Benzim*

The mc
selectivity
zoles appe
moderately
the thiophi
the respect
ment [3]. N
the body li
36, 37] as
neath adh
[5] have b
between 3
erately aci
fluenced b
(table 2). S
across cel
of free p
beyond m

proved to be equipotent in animal experiments. This has been confirmed by Koop et al. [29] and Brunner et al. [30] in healthy volunteers comparing 40-mg doses of either drug upon oral administration and steady-state conditions. In line with this, 20 mg omeprazole were less effective than 40 mg pantoprazole during steady state [31]. Another crossover pH metry study in healthy volunteers [32] confirmed this notion when comparing 20 mg omeprazole and 40 mg pantoprazole. Therefore, the comparison between human data on pantoprazole and omeprazole has been primarily based in this paper on a daily, oral dose of 40 mg each. Like pantoprazole, omeprazole is meanwhile available in various countries in an oral 40-mg dosage. Omeprazole 40 mg daily has been recognized in the meantime as the correct dose offering significant advantages over the 20-mg dose at least in patients with large gastric or duodenal ulcers [33–35].

The Importance of pH-Dependent Activation for Selectivity of Substituted Benzimidazoles: General Considerations

The most important determinant of the selectivity profile of substituted benzimidazoles appears to be the probability to which moderately acidic tissues will be exposed to the thiophilic cyclic sulfenamide formed from the respective prodrug in an acidic environment [3]. Moderately acidic compartments in the body like lysosomes or late endosomes [6, 36, 37] as well as the microenvironment beneath adherent macrophages and osteoclasts [5] have been reported to produce pH values between 3 and 5. Drug exposure of such moderately acidic tissue compartments will be influenced by the AUC of the respective drug (table 2). Since only the free drug equilibrates across cell membranes, the concentration of free prodrug in aqueous compartments beyond membrane barriers will decrease with an increasing percentage of serum protein binding. A higher volume of distribution may be partially explained by a higher logP and will again point to a potentially higher tissue exposure. Once the substituted benzimidazole prodrug has entered the moderately acidic compartment under consideration, it undergoes a pH-dependent intramolecular rearrangement to the thiophilic cyclic sulfenamide that immediately reacts with SH groups in its vicinity to form a covalent bond [for a review, see 3, 4]. Therefore, if a given drug should be activated more quickly at a critical pH relative to its serum elimination half-life than another one, it would generate a higher risk of producing unwanted SH reactions in tissue compartments that achieve this critical pH value. Although concentrations of PPIs necessary to produce unwanted in vitro effects at moderately acidic targets (see below) are high compared to their serum concentrations in man, the former are somewhat arbitrary as they strongly depend on the experimental conditions. Moreover, even minor covalent SH reactions at unwanted targets may add over time to previous injury of these structures under pathological conditions to become manifest. Therefore, the serum AUC rather than maximum concentration values may be important in this context.

The Estimated Tissue Exposure to the Prodrug

Table 2 provides a direct comparison (except for volume of distribution) between pantoprazole and omeprazole for the drug properties mentioned above. The table shows that the different protein binding of the two drugs is already sufficient to reverse the order of AUC values in the serum as far as its significance for drug equilibration across cell membranes is regarded (free drug). This reasoning holds true for both of the total drug concentrations used and is further supported by a high-

er volume of distribution of omeprazole compared to pantoprazole. Irrespective of different drug concentrations within membranes as a consequence of different logP values at pH 7.4, it should be noted that drugs equilibrate across membranes to achieve within the aqueous compartments on either side the same steady-state concentration, i.e. the concentration of free drug present in serum, provided that similar pH values exist on either side. Under this condition, the estimated tissue exposure to the free prodrug will be higher for omeprazole than for pantoprazole. It is the free prodrug in the moderately acidic aqueous tissue compartment that may cause unwanted SH reactions upon its activation.

The Relevance of the Serum Elimination Half-Life Relative to the Prodrug's Activation Half-Life for Selectivity of Substituted Benzimidazoles

The shorter the serum elimination half-life in comparison to the activation half-life of the prodrug at an unwanted target is, the lower the risk of unwanted, covalent SH reactions at this target will be, and vice versa. Table 1 demonstrates that omeprazole is eliminated from serum about as fast as it is activated at a critical pH of about 5, whereas pantoprazole is eliminated from serum by a factor of 2.3–3.8 faster than it is activated at unwanted targets of a pH of 5 or 5.1, respectively.

Biological in vitro Correlates for pH Selectivity

Impracticable large numbers of therapeutic drug administrations are required to make a valid, prospective and comparative assessment of clinical drug safety [38]. Therefore, we have performed in vitro experiments using biological substrates with functional end points in order to detect potential differences between the drugs. Table 3 shows the selectivity factors that indicate the IC_{50} for the sodium pump in multiples of the IC_{50} for the acid pump (histamine stimulation). Clearly, pantoprazole displayed the highest selectivity compared to omeprazole and lansoprazole. A previous comparison in a similar system (acid pump stimulated by db-cAMP [20]) generated IC_{50} values resulting in selectivity factors of 200 (pantoprazole), 100 (lansoprazole) and 60 (omeprazole). It should be noted that the absolute magnitude of any single selectivity factor is meaningless because two completely different experimental models are compared. However, of importance is the rank order of these selectivity factors in the comparison between the different drugs.

The lower IC_{50} value of omeprazole in the fundic gland preparation, compared to pantoprazole (table 3), reflects its faster activation in the acidic compartment of the fundic gland parietal cell in vitro but does not translate into the in vivo animal experiment or into clinical conditions. This is because the intracanalicular pH in vitro is probably higher than in vivo (by about 1–1.5 pH units [W. Beil, pers. commun.]), and the time period available for diffusion, accumulation and activation of the prodrug in the in vitro experiment is limited compared to an open time frame under clinical conditions. Moreover, the faster the drug is activated to immediately block the proton pump, the longer the remaining time fraction in the in vitro experiment available for spontaneous dissipation of the preexisting pH gradient in the particular parietal cell will be. Particularly this latter argument may apply to differences between IC_{50} values found by Beil et al. [39] in pumping membrane vesicles incubated for 40 min, when the pH gradient was indirectly measured by fluorescence quenching of acridine orange. Consequently, these authors interpreted the higher IC_{50} values and the slower time course of the action of pantoprazole as an 'improved' pH stability.

Simon e
acidificatio
kidney is i
concentrati
factor of 2.
zole. Lysos
5 where pa
chemical a
(pH 5) or 4
This comp
experimen
and therefc
centration
acidificati
tal conditi

This no
kanen and
cant inhit
already by
was almos
centration
bone reso
bly, therap
to patients
cal labora
inhibition

Several
prazole ir
both in vi
dall [43] d
reduction
ion genera
instance
graded on
tion', 'aci
3 for 15 m
to polym
in buffer
bation. A
will not r
omeprazc
active m
functions
functions

Simon et al. [16] have shown that luminal acidification of lysosomes prepared from rat kidney is inhibited to 50% by micromolar concentrations of omeprazole that are, by a factor of 2.6, lower than the IC_{50} of pantoprazole. Lysosomes generate a pH_i in the order of 5 where pantoprazole and omeprazole display chemical activation half-lives of 2.8 and 1 h (pH 5) or 4.7 and 1.4 h (pH 5.1), respectively. This compares to an incubation time in the experiment of Simon et al. [16] of only 30 min and therefore explains the relatively high concentrations required to inhibit lysosomal acidification by 50% under these experimental conditions.

This notion is further supported by Tuukkanen and Väänänen [40] who found a significant inhibition of bone resorption in vitro already by 10 μmol/l omeprazole. Inhibition was almost complete at 100 μmol/l. This concentration also completely inhibited in vitro bone resorption in another study [41]. Notably, therapeutic administration of omeprazole to patients resulted in changes in several clinical laboratory parameters that pointed to an inhibition of bone resorption [42].

Several publications have shown that omeprazole interferes with leukocyte functions, both in vitro and in vivo. For example, Wandall [43] described a concentration-dependent reduction in chemotaxis and superoxide anion generation with an IC_{50} value in the latter instance of 250 μmol/l. Although 'acid-degraded omeprazole also inhibited O_2^- generation', 'acid degradation' was performed at pH 3 for 15 min and this material was then added to polymorphonuclear leukocytes suspended in buffer of pH 7.3 for a further 30-min incubation. As seen from table 1, this procedure will not result in a complete degradation of omeprazole and will probably leave sufficient active material for inhibition of leukocyte functions. Hence, inhibition of leukocyte functions by 'degraded' omeprazole does not argue, under Wandall's experimental conditions, against pH-dependent activation in acidic cell compartments as the basis of the observed effects. These experimental conditions may also explain the high drug concentration required.

This principle finding has been confirmed in different test systems demonstrating that omeprazole attenuates oxygen-derived free radical production from neutrophils activated by *Helicobacter pylori* [44], attenuates respiratory burst of human neutrophils by increasing intralysosomal pH [45] and decreases human natural killer cell activity concentration-dependently [46]. Again, 'degraded omeprazole' showed a similar action in the latter instance, but the degradation procedure was performed in a complex medium containing proteins that will slow the degradation rate [Sturm, pers. commun.]. Finally, Scaringi et al. [47] demonstrated in vitro inhibition of cell-mediated cytotoxicity in a dose- and time-dependent manner. More importantly, however, omeprazole 40 mg/day administered for 7 days to healthy volunteers significantly reduced ex vivo chemiluminescence, a measure of the production of reactive oxygen species, in peripheral neutrophils [48]. This confirmed earlier data showing that omeprazole reduces ex vivo production of superoxide anion by polymorphonuclear leukocytes during clinical therapy with omeprazole [49].

Our data on the inhibition of chemiluminescence of fMLP-stimulated human polymorphonuclear leukocytes by 100 μmol/l omeprazole is in line with the above reports. This high drug concentration does not invalidate the biological significance of the inhibitory response since the preincubation time of 5 min was short relative to chronic administration under therapeutic conditions. Leukocytes and macrophages generate reactive oxygen species within lysosomes [50, 51] where they provide moderately acidic conditions for

omeprazole activation. Although our experimental conditions do not allow to distinguish between omeprazole-induced inhibition of either lysosomal or cell membrane NAPH oxidase, the latter activity results from membrane fusion of vesicles and is associated with a proton pump [52]. Thus, there is a proton source at the cell membrane of the activated neutrophil even under the latter conditions. Of relevance in this context is that the activated PPI will be immediately withdrawn from the diffusion equilibrium by its covalent SH reaction. Apart from this cellular effect, about half of the inhibition of chemiluminescence in our system was probably due to antioxidant properties of omeprazole as already described by Lapenna et al. [53] and indicated by 24% inhibition of chemiluminescence in a cell-free hypoxanthine/xanthine oxidase system. By contrast, Wandall [43] did not find any scavenging of O_2^- by omeprazole in a cell-free xanthine/xanthine oxidase system.

The mechanism of action by which omeprazole treatment (20 mg daily for 3 months) in duodenal ulcer patients increased the percentage of HLA-DR-positive peripheral blood lymphocytes as well as random migration, directed migration, phagocytosis index and HLA-DR expression in peripheral blood monocytes-macrophages [54] is unknown. Although both Wandall [43] and Suzuki et al. [48] on the one hand, and Kountouras et al. [54] on the other, discussed the either inhibitory or stimulatory effects in terms of beneficial properties of omeprazole, we consider either 'anti-inflammatory' or 'immunopotentiating' actions of any PPI as unwanted effects. Actually, they indicate effects probably at moderately acidic targets in cells other than the parietal cell.

Acknowledgement

The generation of experimental data by A. Hatzelmann, W.A. Simon and E. Sturm is gratefully acknowledged.

References

1 Hersey SJ, Sachs G: Gastric acid secretion. Physiol Rev 1995;75: 155–189.

2 Huber R, Kohl B, Sachs G, Senn-Bilfinger J, Simon WA, Sturm E: Review article: The continuing development of proton pump inhibitors with particular reference to pantoprazole. Aliment Pharmacol Ther 1995;9:363–378.

3 Kromer W: Similarities and differences in the properties of substituted benzimidazoles: A comparison between pantoprazole and related compounds. Digestion 1995;56: 443–454.

4 Sachs G, Shin JM, Briving C, Wallmark B, Hersey S: The pharmacology of the gastric acid pump: The H^+, K^+-ATPase. Annu Rev Pharmacol Toxicol 1995;35:277–305.

5 Silver IA, Murrills RJ, Etherington DJ: Microelectrode studies on the acid microenvironment beneath adherent macrophages and osteoclasts. Exp Cell Res 1988;175:266–276.

6 Ohkuma S, Poole B: Fluorescence probe measurement of the intralysosomal pH in living cells and the perturbation of pH by various agents. Proc Natl Acad Sci USA 1978;75:3327–3331.

7 Baron R, Neff L, Louvard D, Courtoy PJ: Cell-mediated extracellular acidification and bone resorption: Evidence for a low pH in resorbing lacunae and localization of a 100-kD lysosomal membrane protein at the osteoclast ruffled border. J Cell Biol 1985;101:2210–2222.

8 Forgac M: Regulation of vacuolar acidification; in Clapham DE, Ehrlich BE (eds): Organellar Ion Channels and Transporters. New York, Rockefeller University Press, 1996, pp 121–131.

9 Rainer G, Krüger U, Klemm K: Synthese und physikalisch-chemische Eigenschaften von Lonazolac-Ca, einem neuen Antiphlogistikum/Antirheumatikum. Drug Res 1981; 31:649–655.

10 Weder HG, Schildknecht J, Lutz RA, Kesselring P: Determination of binding parameters from Scatchard plots. Eur J Biochem 1974;42:475–481.

11 Huber R, Müller W, Banks MC, Rogers SJ, Norwood PC, Doyle E: High-performance liquid chromatographic determination of the H^+/K^+-ATPase inhibitor (BY 1023/SK&F

96022) and its sulphone metabolite in serum or plasma by direct injection and fully automated pre-column sample clean-up. J Chromatogr 1990;529:389–401.
12 Sauter R, Steinijans VW, Diletti E, Böhm A, Schulz HU: Presentation of results from bioequivalence studies. Int J Clin Pharmacol Ther Toxicol 1992;30:233–256.
13 Bliesath H, Huber R, Hartmann M, Lühmann R, Wurst W: Dose linearity of the pharmacokinetics of the new H^+/K^+-ATPase inhibitor pantoprazole after single intravenous administration. Int J Clin Pharmacol Ther 1994;32:44–50.
14 Andersson T, Cederberg C, Regardh C-G, Skanberg I: Pharmacokinetics of various single intravenous and oral doses of omeprazole. Eur J Clin Pharmacol 1990;39:195–197.
15 Notari RE: Biopharmaceutics and Clinical Pharmacokinetics: An Introduction, ed 4. New York, Marcel Dekker, 1987.
16 Simon WA, Keeling DJ, Laing SM, Fallowfield C, Taylor AG: BY 1023/SK&F 96022: Biochemistry of a novel ($H^+ + K^+$)-ATPase inhibitor. Biochem Pharmacol 1990;39:1799–1806.
17 Simon WA, Sturm E, Kohl B: High pH selectivity for inhibition of acid secretion in isolated gastric glands by pantoprazole. Digestion 1991;49:52–53.
18 Hatzelmann A, Ullrich V: Regulation of 5-lipoxygenase activity by the glutathione status in human polymorphonuclear leukocytes. Eur J Biochem 1987;169:175–184.
19 Kromer W, Kohl B: Pantoprazole sodium. Drugs Future 1990;15:801–804.
20 Kohl B, Sturm E, Senn-Bilfinger J, Simon WA, Krüger U, Schaefer H, Rainer G, Figala V, Klemm K, Ife RJ, Leach CA: (H^+, K^+)-ATPase inhibiting 2-[(2-pyridylmethyl)sulfinyl]benzimidazoles. 4. A novel series of dimethoxypyridyl-substituted inhibitors with enhanced selectivity: The selection of pantoprazole as a clinical candidate. J Med Chem 1992;35:1049–1057.
21 Fitton A, Wiseman L: Pantoprazole: A review of its pharmacological properties and therapeutic use in acid-related disorders. Drugs 1996;51:460–482.
22 Wilde MI, McTavish D: Omeprazole: An update of its pharmacology and therapeutic use in acid-related disorders. Drugs 1994;48:91–132.
23 Barradell LB, Faulds D, McTavish D: Lansoprazole: A review of its pharmacodynamic and pharmacokinetic properties and its therapeutic efficacy in acid-related disorders. Drugs 1992;44:225–250.
24 Prous Science: E-3810. Drugs Future 1991;16:19–22.
25 Morii M, Takeguchi N: Different biochemical modes of action of two irreversible H^+, K^+-ATPase inhibitors, omeprazole and E3810. J Biol Chem 1993;268:21553–21559.
26 Tomiyama Y, Morii M, Takeguchi N: Specific proton pump inhibitors E3810 and lansoprazole affect the recovery process of gastric secretion in rats differently. Biochem Pharmacol 1994;48:2049–2055.
27 Kromer W, Gönne S, Riedel R, Postius S: Direct comparison between the ulcer-healing effects of two H^+-K^+-ATPase inhibitors, one M_1-selective antimuscarinic and one H_2 receptor antagonist in the rat. Pharmacology 1990;41:333–337.
28 Kromer W, Postius S, Riedel R, Simon WA, Hanauer G, Brand U, Gönne S, Parsons ME: BY 1023/SK&F 96022 INN pantoprazole, a novel gastric proton pump inhibitor, potently inhibits acid secretion but lacks relevant cytochrome P450 interactions. J Pharmacol Exp Ther 1990;254:129–135.
29 Koop H, Kuly S, Flüg M, Schneider A, Rose K: Comparison of 24-h intragastric pH and 24-h gastrin profiles during therapy with the proton pump inhibitors pantoprazole and omeprazole. Gut 1994;35(suppl 4):A79.
30 Brunner G, Danz-Neeff H, Athmann C, Samayoa N: Comparison of pantoprazole (40 mg sid) versus omeprazole (40 mg sid) on intragastric pH and serum gastrin in healthy volunteers. Can J Gastroenterol 1997;11(suppl A):S2.
31 Hartmann M, Theiss U, Huber R, Lühmann R, Bliesath H, Wurst W, Lücker PW: Twenty-four-hour intragastric pH profiles and pharmacokinetics following single and repeated oral administration of the proton pump inhibitor pantoprazole in comparison to omeprazole. Aliment Pharmacol Ther 1996;10:359–366.
32 Scholtz HE, Meyer BH, Luus HG: Comparison of the effects of lansoprazole, omeprazole and pantoprazole on 24-hour gastric pH in healthy males. S Afr Med J 1995;85:915.
33 Valenzuela JE, Kogut DG, McCullough AJ, Colón Pagán JR, Shah U, Whipple J Gilde LR, Simon TJ: Comparison of once-daily doses of omeprazole (40 and 20 mg) and placebo in the treatment of benign gastric ulcer: A multicenter, randomized, double-blind study. Am J Gastroenterol 1996;91:2516–2522.
34 Lauritsen K, Rutgersson K, Bolling E, Brunner G, Eriksson S, Frison L, Galmiche JP, Thomson ABR, Walan A, Archambault AP, Gudjónsson H, Bailey R, Bianchi Porro G: Omeprazole 20 or 40 mg daily for healing of duodenal ulcer? A double-blind comparative study. Eur J Gastroenterol Hepatol 1992;4:995–1000.
35 Müller P, Simon B: Wird Omeprazol richtig dosiert? Münch Med Wochenschr 1996;48:802–804.
36 Myers BM, Prendergast FG, Holman R, Kuntz SM, LaRusso NF: Alterations in the structure, physicochemical properties, and pH of hepatocyte lysosomes in experimental iron overload. J Clin Invest 1991;88:1207–1215.
37 Clague MJ, Urbé S, Aniento F, Gruenberg J: Vacuolar ATPase activity is required for endosomal carrier vesicle formation. J Biol Chem 1994;269:21–24.
38 Peace KE: Design and analysis considerations for safety data, particularly adverse events; in Sogliero-Gilbert G (ed): Drug Safety Assessment in Clinical Trials. New York, Marcel Dekker, 1993, pp 305–316.

39 Beil W, Staar U, Sewing K-Fr: Pantoprazole: A novel H^+/K^+-ATPase inhibitor with an improved pH stability. Eur J Pharmacol 1992;218: 265–271.
40 Tuukkanen J, Väänänen HK: Omeprazole, a specific inhibitor of H^+-K^+-ATPase, inhibits bone resorption in vitro. Calcif Tissue Int 1986; 38:123–125.
41 Zaidi M: Modularity of osteoclast behaviour and 'mode-specific' inhibition of osteoclast function. Biosci Rep 1990;10:547–556.
42 Mizunashi K, Furukawa Y, Katano K, Abe K: Effect of omeprazole, an inhibitor of H^+, K^+-ATPase, on bone resorption in humans. Calcif Tissue Int 1993;53:21–25.
43 Wandall JH: Effects of omeprazole on neutrophil chemotaxis, superoxide production, degranulation, and translocation of cytochrome b_{-245}. Gut 1992;33:617–621.
44 Suzuki M, Mori M, Suzuki H, Asako H, Fukumura D, Miura S, Tsuchiya M: Omeprazole attenuates oxygen-derived free radical production from neutrophils activated by *Helicobacter pylori*. Immunol Microbiol Inflamm Dis 1993;104:A787.
45 Suzuki M, Mori M, Fukumura D, Suematsu M, Miura S, Tsuchiya M: Omeprazole attenuates respiratory burst of human neutrophil by increasing intralysosomal pH. Immunol Microbiol Inflamm Dis 1994; 106:A779.
46 Aybay C, Imir T, Okur H: The effect of omeprazole on human natural killer cell activity. Gen Pharmacol 1995;26:1413–1418.
47 Scaringi L, Cornacchione P, Fettucciari K, Rosati E, Rossi R, Marconi P, Capodicasa E: Activity inhibition of cytolytic lymphocytes by omeprazole. Scand J Immunol 1996;44: 204–214.
48 Suzuki M, Mori M, Miura S, Suematsu M, Fukumura D, Kimura H, Ishii H: Omeprazole attenuates oxygen-derived free radical production from human neutrophils. Free Radical Biol Med 1996;21:727–731.
49 Capodicasa E, Ciaccio V, Corazzi F, De Bellis F, Bucaneve G, Ficola F, Pelli MA: Reduced 'ex-vivo' production of superoxide anion by polymorphonuclear leukocytes during therapy with omeprazole: A pharmacologic effect? Minerva Gastroenterol Dietol 1994;40:105–111.
50 Roos D: The involvement of oxygen radicals in microbicidal mechanisms of leukocytes and macrophages. Klin Wochenschr 1991;69: 975–980.
51 Rosen GM, Pou S, Ramos CL, Cohen MS, Britigan BE: Free radicals and phagocytic cells. FASEB J 1995; 9:200–209.
52 Watson F, Robinson JJ, Edwards SW: Sequential phospholipase activation in the stimulation of the neutrophil NADPH oxidase. FEMS Microbiol Immunol 1992;105:239–248.
53 Lapenna D, de Gioia S, Ciofani G, Festi D, Cuccurullo F: Antioxidant properties of omeprazole. FEBS Lett 1996;382:189–192.
54 Kountouras J, Boura P, Apostolides G, Zaharioudaki E, Tsapas G: In vivo effect of omeprazole on HLA-DR expression and the monocyte-macrophage function in patients with duodenal ulcer disease. Immunopharmacol Immunotoxicol 1994; 16:437–448.
55 Yasuda S, Ohnishi A, Ogawa T, Tomono Y, Hasegawa J, Nakai H, Shimamura Y, Morishita N: Pharmacokinetic properties of E3810, a new proton pump inhibitor, in healthy male volunteers. Int J Clin Pharmacol Ther 1994;32:466–473.

#197

Evaluation of Buffering Capacity and Acid Neutralizing-pH Time Profile of Antacids

Mei-Shu Lin, Pin Sun, and Hsiu-Ying Yu

Abstract The antacid properties of seven antacids listed in the hospital formulary of a medical center were evaluated with *in vitro* tests. These included not only the preliminary antacid test and acid-neutralizing capacity test as described in the United States Pharmacopeia (USP XXIII), but also a buffering pH profile test. The preliminary antacid test measured the final pH of a 10-mL solution of 0.5 N HCl 10 minutes after addition of the minimum recommended dose of an antacid, while the neutralizing capacity test measured the amount (mEq) of HCl neutralized by the minimum recommended dose in 15 minutes. The buffering pH profile recorded the pH time course of dynamic simulated gastric fluid neutralization by a dose of an antacid. In the preliminary antacid test, magnesium oxide showed the highest pH (9.52 ± 0.14, mean ± standard deviation, n = 3); aluminum phosphate gel yielded a final pH of 2.51 ± 0.01, thus failing to meet the criteria of an antacid (pH > 3.5). In the acid-neutralizing capacity test, hydrotalcite had the highest neutralizing capacity (28.26 ± 0.3 mEq), while sodium bicarbonate had the lowest (7.40 ± 0.12 mEq). In the buffering pH profile test, aluminum-magnesium hydroxide suspensions and hydrotalcite tablets maintained a steady optimum pH (3–5) for around 1.5 hours. One tablet of calcium carbonate, sodium bicarbonate or magnesium oxide could not raise the gastric pH to above 3, but two tablets increased the pH excessively (5.3 to 8.6). The higher dose (two tablets) of aluminum hydroxide hexitol complex could not raise the pH to the optimal level. These findings demonstrate that there is disparity in the antacid effectiveness estimated by the neutralizing capacity test and the buffering pH profile test and suggest that the efficacy of an antacid cannot be accurately predicted from its acid-neutralizing capacity. The dose of antacids greatly influences the neutralizing pH profiles. Aluminum-magnesium compounds appear to provide steadier buffering than carbonate compounds or magnesium oxide.

(*J Formos Med Assoc* 1998;97:704–10)

Key words:
antacid
buffering capacity
pH
neutralization

It is generally agreed that an ideal antacid should possess at least the following characteristics: rapid neutralization, in order to quickly relieve the discomfort caused by acidic gastric juice, and to cope with the rate of gastric emptying [1]; acid-neutralizing capacity sufficient to achieve the optimum pH (3–5) [2] without over-neutralizing, in order to avoid a rebound effect [3]; long-lasting antacid effect; and antacid activity unaffected by digestive enzymes.

The method for determining antacid effectiveness in the United States Pharmacopeia (USP) XXIII includes two tests: the preliminary antacid test and the acid-neutralizing capacity test [4]. In the preliminary antacid test, the acid-neutralizing ability of an antacid is evaluated by mixing the minimum recommended dose of the antacid with 10 mL of 0.5 N HCl for 10 minutes. The final pH of the reaction mixture is then measured, and should be 3.5 or greater. The acid-neutralizing capacity test measures the amount of HCl neutralized by the minimum recommended dose of an antacid. The antacid is mixed with an excess of 1.0 N HCl at 25°C or 37°C for exactly 15 minutes, and the excess acid is then back-titrated with 0.5 N NaOH in a period not exceeding 5 minutes. The acid-neutralizing capacity test does not take into account the time required for acid neutralization or the duration of buffering by an antacid; it is merely

School of Pharmacy, College of Medicine, National Taiwan University, Taipei.
Received: 4 May 1998. Revised: 4 June 1998. Accepted: 4 August 1998.
Reprint requests and correspondence to: Professor Hsiu-Ying Yu, School of Pharmacy, College of Medicine, National Taiwan University, 1 Jen-Ai Road, Section 1, Taipei 100, Taiwan.

a measure of excess acid neutralized at 15 minutes. Because the relief of pain due to excess gastric acidity is probably because of the change in pH of the gastric contents [3], physicians and patients are more interested in the rapidity and duration of neutralization than in the total amount of acid that can be neutralized over a period of 15 minutes. Importantly, these two tests do not show the rate of change in pH, which is related to the clinical effectiveness of antacids.

A variety of dynamic *in vitro* tests have been designed to correlate more closely with conditions likely to be encountered in general use of such compounds in clinical practice. The Rossett-Rice test [5] is one such method for characterization and evaluation of antacid compounds [6–9].

In our study, the antacid efficacies of seven common antacids that are included in the hospital formulary of National Taiwan University Hospital were evaluated *in vitro* with the antacid test, the acid-neutralizing capacity test, and a modification of the Rossett-Rice acid-neutralizing pH-time profile test, which incorporates simulated gastric secretion. We compared the results of the static and the dynamic neutralizing experiments to investigate the differences in acid-neutralizing activity among various antacids. The results should be helpful in the clinical selection of appropriate antacids for optimal treatment, as well as in the interpretation of antacid tests.

Materials and Methods

Seven commercially available antacids listed in the hospital formulary of the National Taiwan University Hospital (Table 1) were obtained from the Department of Pharmacy, National Taiwan University Hospital. Two of the antacid preparations were colloidal suspensions and the other five were tablets.

Each antacid was evaluated with the preliminary antacid test and the acid-neutralizing capacity test according to the United States Pharmacopeia (USP) XXIII guidelines [4]. In the acid-neutralizing test, antacids were comminuted sufficiently to pass through a number 20 sieve (Ming-Shiang, Taipei, Taiwan). The sieved material was then accurately weighed, and an amount equivalent to the minimum recommended dosage was dissolved in distilled water in a final volume of 40 mL by mixing in a beaker with a magnetic stirrer (Thermolyne, Dubugue, IO, USA) at 300 rpm for 1 minute. For liquid antacids, the minimum recommended dosage was accurately weighed and mixed in water to a final volume of 40 mL. Then, 10.0 mL of 0.5 N HCl was added to the test solution, with stirring. Exactly 10 minutes after the addition of the acid, the pH of the reaction mixture was read and recorded. If the pH was below 3.5, the product was not considered an antacid and it was not included in the other tests. If the pH was 3.5 or greater, the antacid was included in the neutralizing capacity test.

The acid-neutralizing capacity test is used to determine the amount (mEq) of acid neutralized by the minimum recommended dosage of the tested antacid. Antacid tablets were comminuted, sieved, and dissolved in water by mixing with a magnetic stirrer at 300 rpm for 1 minute, and the final volume was adjusted to 70 mL. Liquid antacids were weighed and used directly. Then, 30.0 mL of 1.0 N HCl was added to the test solution with stirring. Exactly 15 minutes after the addition of acid, the excess acid in the test solution was titrated with 0.5 N NaOH to a stable pH of 3.5. All tests were conducted at 37 ± 3°C (25 ± 3°C was also allowed) [4]. The amount of acid neutralized by the antacid sample was calculated as

Table 1. Antacids tested

Antacid	Chemical formula	Form	Content/dosage unit	Minimum recommended dose
Aluminum-magnesium hydroxide	$AlMg_2(OH)_7$	Suspension	1.5 g in 20-mL sachet	798 mg (1/2 sachet)
Aluminum phosphate	$AlPO_4$	Suspension	2.5 g in 26-g sachet	2.5 g (1 sachet)
Aluminum hydroxide hexitol complex	$Al(OH)_3$	Tablet	233 mg	233 mg (1 tablet)
Hydrotalcite	$Al_2Mg_6(OH)_{16}CO_3 \cdot 4H_2O$	Tablet	0.5 g	1 g (2 tablets)
Calcium carbonate	$CaCO_3$	Tablet	0.5 g	1 g (2 tablets)
Sodium bicarbonate	$NaHCO_3$	Tablet	600 mg	600 mg (1 tablet)
Magnesium oxide	MgO	Tablet	250 mg	400 mg (1.6 tablets)

follows: Total mEq = (30.0 mL) (normality of HCl) – (mL of NaOH) (normality of NaOH).

The pH-time profile during the neutralization reaction was determined using the method of Rossett-Rice [5], with modifications as previously described [7,9]. In brief, the system consisted of a beaker containing 150 mL (approximate fasting content of human stomach) [1] of simulated gastric fluid. Simulated gastric fluid test solution was prepared according to the USP XXIII guidelines [10]. Briefly, 2.0 g of NaCl and 3.2 g of pepsin (E Merck, Darmstadt, Germany) were dissolved in 7.0 mL of HCl, and the volume was adjusted to 1,000 mL with distilled water. The pH of this test solution was 1.2. The beaker was placed on a thermostatic magnetic stirrer to keep the contents at 37 ± 1°C and to provide continuous stirring (300 ± 30 rpm). A pH electrode was kept in the upper region of the solution. The antacid to be tested was added into the beaker to start the neutralization reaction. After the first 10 minutes, fresh simulated gastric fluid was delivered into the reaction vessel via a tube, to simulate gastric secretion. The delivery of the simulated gastric fluid was controlled with a peristaltic pump at a constant rate of 1.6 mL/minute, and reaction solution was drained via another tube to keep the volume of the reaction solution constant. Because some antacid tablets can be swallowed whole or chewed, the pH-profile experiments for antacid tablets were conducted with both whole tablets and comminuted tablets [4]. The pH-time profile of the reaction mixture was recorded for 120 minutes. The weight variation of the antacid tablets and suspensions were determined according to USP XXIII guidelines [11].

Results

The results of the preliminary antacid test and the acid-neutralizing capacity test are shown in Table 2. In the preliminary antacid test, aluminum phosphate yielded a final pH of 2.51 ± 0.01, well below the 3.5 cut-off value for an antacid [4], and was not considered an antacid. Therefore, aluminum phosphate was excluded from further experiments. Among the qualified antacids, the final pH achieved by the minimum recommended dose was the highest for magnesium oxide and the lowest for aluminum hydroxide hexitol complex (Table 2).

The acid-neutralizing capacity per minimum recommended dose of antacid (Table 2) was the lowest for sodium bicarbonate and the highest for hydrotalcite. When normalized according to weight, the acid-neutralizing capacity per gram of active constituent was lowest for sodium bicarbonate, and highest for magnesium oxide. The values of acid-neutralizing capacity are in agreement with those of effective antacids contained in the minimum recommended dose (Table 2).

The pH-profile test (Figure) demonstrated that all the tested whole-tablet antacids except magnesium oxide had rapid onset of neutralizing effect (less than 5 minutes), with a peak pH appearing in 10 minutes or less. The pH profiles were different between the low (one tablet or 1/2 sachet) and high (two tablets or one sachet) doses. The high dose yielded a significantly ($p < 0.05$) higher maximum pH and a longer duration of effective buffering than the low dose for all the tested antacids (Table 3). The pH profiles of the whole-tablet and the powdered form of antacids were almost identical, except for magnesium oxide. The powdered form of antacids showed more rapid onset of neutralization.

The pH profiles showed that the buffering action of aluminum hydroxide compounds, including aluminum-magnesium hydroxide, aluminum hydroxide hexitol complex, and hydrotalcite (Figure A, B, and C), were moderate. The high dose of aluminum-magnesium hydroxide compounds maintained a steady effective pH (4–5 or 3–4) (Figure A and C) for a period of more than 1 hour. However, when the low dose of the same compounds was used, the pH gradually declined to below 3.0 in less than 30 minutes after the peak pH was achieved.

Table 2. Results of preliminary antacid test and acid-neutralizing capacity test of antacids

Antacid	PAT	Acid-neutralizing capacity	
	Final pH	mEq HCl per minimum dose*	mEq HCl/g
Aluminum-magnesium hydroxide	5.72 ± 0.13	25.75 ± 0.19 (27)†	34.89 ± 0.26
Aluminum phosphate	2.51 ± 0.01	Not tested	Not tested
Aluminum hydroxide hexitol complex	3.65 ± 0.05	9.28 + 0.13 (9.0)†	39.83 ± 0.56
Hydrotalcite	5.42 ± 0.21	28.26 ± 0.30 (26.5)†	28.26 ± 0.30
Calcium carbonate	6.13 ± 0.13	20.39 ± 0.08 (20.0)†	20.39 ± 0.08
Sodium bicarbonate	7.41 ± 0.11	7.40 ± 0.12 (7.1)†	12.33 ± 0.20
Magnesium oxide	9.52 ± 0.14	18.98 ± 0.26 (19.8)†	47.45 ± 0.65

PAT = pH-time antacid test [4]. *Minimum recommended dose. †Numbers in parentheses are minimum recommended doses of antacids in mEq. Data are mean ± SD of three determinations.

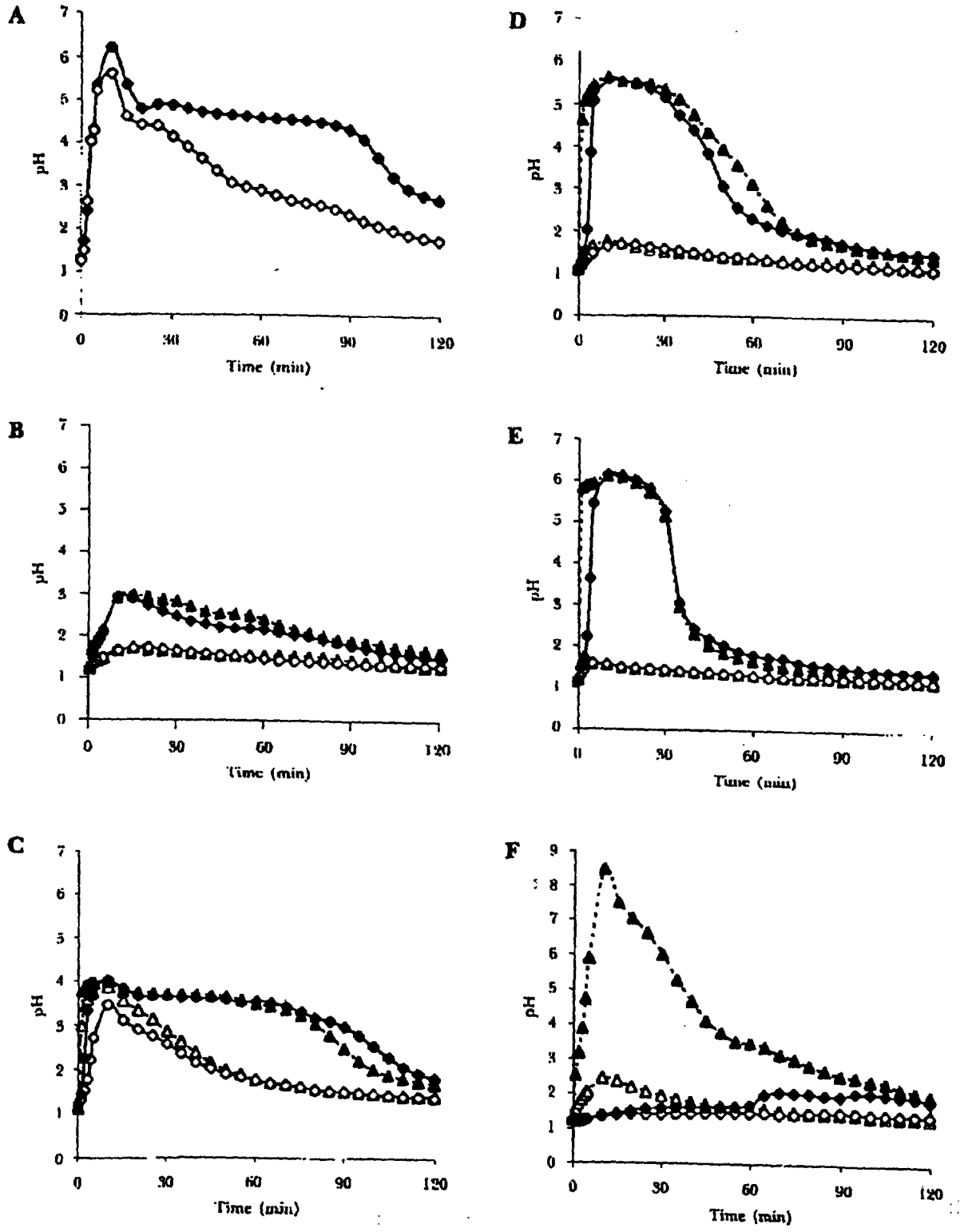


Figure. *The pH profiles of antacids assessed with a modification of the Rossett and Rice method [5]. A) aluminum-magnesium hydroxide suspension; B) aluminum hydroxide hexitol complex; C) hydrotalcite; D) calcium carbonate; E) sodium bicarbonate; F) magnesium oxide. The curves shown are plotted from the results of one representative pH-profile test for each sample. (-◆-) 20 mL suspension (A) or two whole tablets, (-◇-) 10 mL suspension (A) or one whole tablet, (-▲-) two tablet powder, (-△-) one tablet powder.*

Table 3. Antacid pH profiles

Antacid	Dose	Maximum pH*	Duration of antacid effect (min)†
Aluminum-magnesium hydroxide	10 mL	5.52 ± 0.22‡	42 (3rd–45th)
	20 mL	6.21 ± 0.05	102 (3rd–105th)
Aluminum hydroxide hexitol complex	1 tablet (T)	1.56 ± 0.07‡	—
	1 tablet (P)	1.68	—
	2 tablets (T)	2.37 ± 0.11	—
	2 tablets (P)	2.95	—
Hydrotalcite	1 tablet (T)	3.70 ± 0.19‡	13 (2nd–15th)
	1 tablet (P)	3.85	23 (2nd–25th)
	2 tablets (T)	4.06 ± 0.02	84 (1st–85th)
	2 tablets (P)	3.98	79 (1st–80th)
Calcium carbonate	1 tablet (T)	1.14 ± 0.04‡	—
	1 tablet (P)	1.84	—
	2 tablets (T)	5.27 ± 0.12	45 (5th–50th)
	2 tablets (P)	5.72	54 (1st–55th)
Sodium bicarbonate	1 tablet (T)	1.51 ± 0.09‡	—
	1 tablet (P)	1.58	—
	2 tablets (T)	6.04 ± 0.32	27 (3rd–30th)
	2 tablets (P)	6.09	29 (1st–30th)
Magnesium oxide	1 tablet (T)	1.56 ± 0.10‡	—
	1 tablet (P)	2.43	—
	2 tablets (T)	2.85 ± 0.11	—
	2 tablets (P)	8.57	73 (2nd–75th)

*Mean ± SD of three determinations for (T), and one determination for (P). †Duration above pH 3 after addition of antacid. ‡Significantly different between high and low doses (Student's t test, $p < 0.5$). T = tablet form, P = powdered form.

The carbonates, $CaCO_3$ and $NaHCO_3$, (Figure D and E) in low doses were not capable of raising the pH to above 2.0 in the pH-profile test and, thus, their antacid properties are dubious. However, the high dose of these antacids exerted strong neutralizing effects (the maximum pH for $NaHCO_3$ and $CaCO_3$ were 6.1 and 5.7, respectively). The neutralizing effect of $NaHCO_3$ was vigorous: the pH rose to 6.1 immediately after addition of the antacid, and remained at above 5 for about 30 minutes, and then fell to below 3 in the next 5 minutes.

The pH profiles of magnesium oxide showed greatly with both the dose and form (powdered vs tablet). The higher recommended dose of magnesium oxide when given in tablet form did not raise the pH to an effective therapeutic level, but the same dose of powdered magnesium oxide showed the highest maximum pH among all the tested antacids in this experiment (Table 3).

The weight variations of the antacid samples are shown in Table 4. All the antacid tablet samples met the USP requirement (85.0%–115.0% of the recommended claim, relative standard deviation 6.0%), but the aluminum-magnesium hydroxide suspension showed a relative standard deviation (20.87%) larger than 6.0% (Table 4).

Table 4. Weight variation of antacids

Composition	Weight variation*	RSD (%)	Range
Combined hydroxides of magnesium and aluminum	20.253 ± 4.227 mL	20.87	15.100 – 23.459 mL
Hydrotalcite	0.941 ± 0.007 g	0.78	0.935 – 0.958 g
Aluminum hydroxide	0.653 ± 0.010 g	1.48	0.637 – 0.668 g
Calcium carbonate	0.604 ± 0.007 g	1.11	0.595 – 0.613 g
Sodium bicarbonate	0.669 ± 0.015 g	2.24	0.633 – 0.687 g
Magnesium oxide	0.470 ± 0.005 g	0.99	0.461 – 0.476 g

RSD = relative standard deviation. *Data are mean ± standard deviation of 10 tablets or sachets.

Discussion

The volume of gastric juice was chosen as 150 mL because it seems a good approximation of the amount of gastric juice present in the resting stomach [1]. The constant replacement of reaction mixture with fresh gastric juice provides a system where the amount of antacid is gradually diminishing while the supply of fresh acid is constant. This *in vitro* procedure may not be completely representative of the behavior of an antacid in the human stomach, but it is perhaps the most severe method of comparing antacids that can be devised under conditions similar to those found in the stomach.

The pH profile shows how fast and how well antacids neutralize excess acid. An antacid should promptly neutralize gastric acid to a pH of 3.5 to 5. Most of the antacids tested neutralized the acid to this level rapidly, but some (aluminum hydroxide hexitol compound and the minimum recommended dose of sodium bicarbonates) did not (Figure). The pH profiles (Figure) show that two tablets of sodium bicarbonate or powdered magnesium oxide resulted in an excessively basic pH (> 6). The sudden drop in pH which followed the excessive pH increase would consequently be expected to cause a rebound effect *in vivo*.

The results of the acid-neutralizing capacity test (USP) did not correlate with those of the pH-profile test. In spite of the relatively small difference in acid-neutralizing capacity (mEq HCl/minimum recommended dose) between aluminum hydroxide hexitol complex (9.28 ± 0.13) and sodium bicarbonate (7.40 ± 0.12), their pH profiles were quite different. Although the acid-neutralizing capacity of aluminum hydroxide hexitol complex was higher than that of sodium bicarbonate, the maximum pH achieved in the pH-profile test of aluminum hydroxide hexitol complex (2 tablets) was only half that of sodium bicarbonate (2 tablets). Aluminum hydroxide hexitol complex fulfilled the USP criteria of an antacid, but it failed to demonstrate antacid efficacy (pH > 3) in the pH-profile test, in which gastric secretion was simulated. The recommended dose of aluminum hydroxide hexitol complex is thus likely to have little effect in patients suffering from excess gastric acidity. Sodium bicarbonate showed the smallest acid-neutralizing capacity, but its maximum pH in the pH profile was higher than that of most antacids. The pH profile results imply that the minimum recommended dose of most antacids is too low.

The pH profiles of the whole-tablet and tablet-powder forms of magnesium oxide were quite different. The maximum pH in the pH-profile test was lower than pH 3 when two whole magnesium oxide tablets were used, but nearly 9 when two powdered tablets were used; this pH is excessively basic. Such a difference is due to slow disintegration of magnesium oxide tablets in the simulated gastric fluid. It is recommended that antacid tablets should be thoroughly chewed before swallowing [12].

It is interesting to note that aluminum hydroxide showed a blunt pH profile (Figure B). However, a combination of magnesium and aluminum hydroxide antacids demonstrated a two-phase pH-profile, an initial peak followed by a steady phase (Figure A and C). The peak pH most likely represents the action of magnesium hydroxide component; magnesium oxide alone demonstrated a sharp and early peak (Figure F). In water, magnesium oxide is converted to magnesium hydroxide and has essentially the same acid-neutralizing effect as magnesium hydroxide. Magnesium oxide reacts rapidly with acid and raises the pH to over 8. The steady pH following the peak represents the action of aluminum hydroxide. Thus, in a combined aluminum-magnesium antacid, the magnesium compound reacts first with acid and raises the pH higher than necessary, while the more slowly reacting aluminum component needs more time before it can elevate the pH to about 4.

The characteristics of the pH profiles of antacids may be approximately predicted from their active components rather than their acid-neutralizing capacity. Aluminum hydroxide compounds react relatively slowly and steadily, carbonates and magnesium compounds react rapidly and vigorously, and the aluminum-magnesium hydroxide compounds demonstrate the combined actions of the two components.

In conclusion, antacid efficacy cannot be properly predicted simply from the acid-neutralizing capacity required by the USP. The dose of antacids greatly influences the pH profile. Aluminum-magnesium compounds provide steadier buffering than carbonate compounds or magnesium oxide. This study also indicates that when antacids are prescribed, the dosage form and dose should be considered.

References

1. Holbert JM, Noble N, Grote IW: A study of antacid buffers: I. The time factor in neutralization of gastric acidity. *J Am Pharm Assoc, Sci Ed* 1947;36:149–51.
2. Keyrilainen O: Methods for the assessment of antacids. *In vitro* and *in vivo* investigations with gastric aspiration. *Scand J Gastroenterol* 1982;17(Suppl 75): 40–3.
3. Pilbrant A: Principles for development of antacids. *Scand J Gastroenterol* 1982;17(Suppl 75):32–6.

4. The United States Pharmacopeia, 23rd Revision, United States Pharmacopeia Convention, Inc., Rockville, MD, 1995:1845–6.
5. Rossett NE, Marion L: An *in vitro* evaluation of the efficacy of the more frequently used antacids with particular attention to tablets. *Antacids* 1954;26: 490–5.
6. Beekman SM: Preparation and properties of new gastric antacids I: Aluminum hydroxide-magnesium carbonate dried gels. *J Am Pharm Assoc* 1960;49:191–200.
7. Beneyto JF, Fabregas JL, Moragues J, et al: Evaluation of a new antacid, almagate. *Arzneim-Forsch/Drug Res* 1984;34 (10A):1350–4.
8. Beckman SM and Vogel CH: Preparation and properties of new gastric antacids II: aluminum hydroxide-protein dried gels. *J Am Pharm Assoc* 1960;49:201–4.
9. Kerkhof NJ, Vanderlaan RK, White JL, et al: pH-Stat titration of aluminum hydroxide Gel. *J Pharm Sci* 1977;66:1528–32.
10. The United States Pharmacopeia, 23rd Revision, United States Pharmacopeia Convention, Inc., Rockville, MD, 1995:2053.
11. The United States Pharmacopeia, 23rd Revision, United States Pharmacopeia Convention, Inc., Rockville, MD, 1995:1838–9.
12. McEvoy GK, Litvak K, eds. *American Hospital Formulary System Drug Information*. Bethesda, MD: American Society of Health-System Pharmacists, 1994;56:1870–3.